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VIA EMAIL

September 1, 2004

Dr. Deborah Drechsler
Air Resources Board
Post Office Box 2815
Sacramento, CA 95812

RE: EMA Comments on the ARB/OEHHA Draft Report on the Ozone Ambient Air Quality Standard, June 2004

Dear Dr. Drechsler:

The Engine Manufacturers Association (EMA) is the trade association representing the major manufacturers of internal combustion engines. Our 28 member companies produce engines that are used in a wide variety of applications including heavy duty on-highway trucks and buses, nonroad farm and construction equipment, locomotives, lawn and garden equipment, marine vessels, and stationary sources such as emergency and prime power electrical generators.

EMA represents member companies on issues related to emissions and air quality and has a long history of working with the California Air Resources Board and the United States Environmental Protection Agency to reduce emissions from engines. In many cases, NOx, PM, and hydrocarbon emissions have already have been reduced by 90 percent, and in the case of new on-highway and nonroad heavy duty engines, manufacturers are committed to meeting an additional 90 percent reduction in the coming years.

As part of the solution to reducing emissions, Engine manufacturers recognize the importance of setting clearly defined, appropriate and science-based goals for cleaner air through the development of ambient air quality standards. Consequently, EMA has reviewed California's Draft Report on the Ozone Ambient Air Quality Standard (Report) and has prepared the following comments for your consideration and incorporation into the final report and presentation to the Board.

EMA's comments consist of the following:

1. The attached report prepared by Sciences International, Alexandria, Virginia, which provides a critical review of the scientific basis for the ozone standards and the scientific literature regarding health effects of ozone exposure.

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2. Several additional comments prepared by EMA staff that generally follow EMA's oral comments provided at the August 25th Workshop.

Based on the review of the Report completed by the toxicologists and epidemiologists of Sciences International as well as our own critique, EMA believes that the analysis and justification regarding the ozone standards presented in the draft report need to be improved. Specifically, the draft report does not adequately address several key factors, and some of the report's statements and conclusions do not appear to be justified by the current scientific literature. EMA asks that the following points be addressed in the final report, and that ARB and OEHHA staff reconsider the proposed ambient air quality standards for ozone based upon a more critical review of the available scientific evidence.

1. The Report needs to better address and evaluate whether the results of human exposure studies which serve as the primary basis for the proposed standards actually meet the criteria as adverse health effects established by the American Thoracic Society.

As the Report properly points out, a key issue in evaluating the results of human health studies is to define an adverse health effect. The Report references the criteria for adverse health effects established by the American Thoracic Society as a necessary first step in documenting or addressing whether ozone exposure produces adverse health effects. However, there is little discussion or analysis as to whether the results of the human ozone exposure studies actually meet the ATS criteria. In fact, when considering whether results of human exposure studies meet the criteria, the Report simply states "many health outcomes found to be associated with ozone could be considered adverse including . . .(Page 8-4, Section 8.2)." In addition, Section 8.3.1.9 that discusses ozone concentrations where adverse effects have been observed again simply states that "many outcomes found to be associated with ozone in chamber studies could be considered adverse . . . outcomes such as an increase in airway reactivity and inflammation may be considered adverse," but again there is no analysis or demonstration that the outcomes observed or measured in chamber studies actually meet the criteria.

At the end of the above-referenced paragraph, the Report makes the statement that "for asthmatics, a repeated decrease in FEV_1 of 20 -30% could necessitate medical intervention . . . which clearly qualifies as an adverse effect." However, there is no information to indicate that a 20-30% decrement in the lung function of asthmatics occurred during testing.

The Report needs to provide a more comprehensive review of the human exposure study results in relation to the criteria chosen to evaluate adverse effects. Many of the effects observed in the human exposure studies are transitory in nature and relatively small. The report needs to carefully evaluate any observed changes and make clear statements of fact as to whether the changes do indeed meet the ATS criteria.

2. Since the Report uses the results of the human exposure/chamber studies as the basis for the proposed ozone standards, a more thorough and critical evaluation of these studies is needed.

ARB and OEHHA rely on the results of the human exposure/chamber studies to establish the scientific basis for the proposed ozone standards. The Report describes the results of the human exposure studies but provides little information or critical analysis of them. There should be more discussion regarding the design, limitations, and short-comings of the studies as well as a comparison of the results so that the public has adequate information to judge the importance and validity of the results reported. For instance, questions arise concerning the design of the studies with regard to undue physiological stress of the subjects, measurement and form of the ozone exposure, possible subject response bias, statistical analysis methods, applicability of the results to the overall population, and differences among studies. These issues need to be fully addressed in the final report.

3. In developing the rationale for the proposed standards, the Report correctly places less emphasis on the epidemiology results due to the inherent uncertainties in such studies, but the Report does not adequately convey the caveats or conflicting results contained in the epidemiology literature on ozone.

The Report is careful to indicate that the proposed standards are based on the human exposure data and uses the epidemiology literature to support the basic arguments surrounding human health effects. The Report also provides the reader with some cautions regarding the scientific difficulties associated with epidemiology studies of air pollution effects. Such epidemiology studies generally cannot discriminate effects of one air pollutant compared to another since the ambient concentrations of many air pollutants are highly correlated.

The final report should contain a better and more thorough discussion of these issues, particularly the confounding issue and the often conflicting results observed in different epidemiology studies. As more thoroughly discussed in the Sciences International Report, some of the results cited as evidence supporting an ozone health effect are not statistically significant, contain both positive and negative results, and include studies where there were stronger signals for an overall air pollution association, but no specific ozone association. The final report should emphasize the fact that many of the ozone studies could not adequately control for, or rule out, effects of confounding.

4. A more thorough and complete discussion of the literature on the effects of ozone on susceptible populations including children and asthmatics needs to be included in the final report.

The Report states that children are more sensitive or may be adversely impacted by ozone exposure to a greater degree than the rest of the population. The Report recognizes some factors associated with children such as an increased outdoor activity and, therefore a potentially higher ozone exposure, smaller lung size, and developmental growth as factors to back up this statement. It is critical to consider whether children are more severely impacted, however, the information provided in the Report is not adequate to document any enhanced effects on

children. The discussion regarding any differential adverse effects on children's health, as well as asthmatics, should be reviewed for completeness and changed accordingly. The Report's concluding statement on this subject does not appear to be well documented by the information presented.

For example, Section 8.4 discusses the evidence for children's health effects but primarily cites animal studies and a single longitudinal epidemiology study as evidence of enhanced effects. However, on Page 8-9 of the Report the statement is made that "the few data available do not identify children or adolescents being more or less responsive than young adults who have undergone similar exposure protocols, although children tend to report fewer symptoms." Some epidemiology studies such as McConnell et al. 2003 and Peters et al. 1999 indicate that the strongest association with children's health effects was with other pollutants rather than ozone or that no association of symptoms with ozone were noted in the children studied. And, even in the McConnell et al. 2002 study where an association with ozone was reported for children playing more than 3 sports, the authors indicate that the overall risk of developing asthma was not higher in the six high pollution communities and that there were some indications of a decreased asthma association with high daily maximum ozone levels.

The section on children's health needs to be re-examined in light of the information available in the scientific literature and referenced in the Report.

5. The report needs to assess and address the impacts on human health from historical and documented reductions in ozone levels and discuss the implications of the results.

The basis for the proposed ozone standards is that certain laboratory exposure studies and limited epidemiology evidence suggests that current ozone levels have an effect on human health and that lower ozone levels should result in less disease and decreased morbidity and mortality.

One key discussion that is missing from the Report is any mention or analysis of documented health changes that have resulted from previous declines in ambient levels of ozone in the state. Air quality in California has improved greatly over the last thirty years with a documented reduction in both peak and annual ozone concentrations. It would be instructive to look at health statistics and effects that resulted from these reductions in ambient ozone concentrations. It follows logically that if there are observable health effects stemming from ambient ozone concentrations, the improvement in health statistics regarding asthma, respiratory impacts and other potential effects should be evident in the historical data comparing indices from times of high ozone levels to today's much lower levels. If this cannot be shown, then the justification and need for future reductions in ambient ozone standards must be questioned.

For example, air quality data reported by the South Coast Air Quality Management District indicate that there has been a very significant decrease in ambient ozone concentrations in the basin over the last 20 years. Exceedence days over the federal 1-hour ozone standard have decreased from around 170 in 1980 to less than 50 in 2001. Similarly, South Coast AQMD trend charts indicate that maximum 1-hour ozone concentration has declined from 0.50 ppm to less than 0.20 ppm over the same time period. If ozone is truly a causal source of health effects, such

dramatic declines in ambient ozone concentrations should have produced similarly significant improvements in health for those living in the basin.

The report does not document or address this key issue. Such a discussion and analysis should be included in the final report and would serve as an important verification of the health effects estimates suggested in the current Report.

EMA appreciates the opportunity to provide comments on the draft report. Please feel free to contact me if you have any questions or would like additional information or clarifications regarding our submittal.

Respectfully submitted,

Joseph L. Suchecki

Joseph L. Suchecki
Director, Public Affairs

COMMENTS ON THE "REVIEW OF THE CALIFORNIA AMBIENT AIRBORNE STANDARD FOR OZONE"

Prepared for

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By

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September 1, 2004

Comments on the "Review of the California Ambient Air Quality Standard for Ozone"

This report presents the comments of Sciences International, Inc. on the June 21, 2004 Public Review Draft of "Review of the California Ambient Air Quality Standard for Ozone" prepared by the Air Resources Board and Office of Environmental Health and Hazard Assessment, California Environmental Protection Agency (referred to henceforth as the Document).

Our report is organized as follows. First, we discuss the controlled human exposure, epidemiology, and animal studies cited in the Document that we consider key to California's proposed standard. This is followed by a discussion of the evidence bearing on the response of asthmatics, children and allergenic people to ozone exposure. The report concludes with a summary of our findings.

CONTROLLED HUMAN EXPOSURE STUDIES

Controlled human exposure studies for ozone consist primarily of chamber studies where volunteers are placed in a chamber and exposed to different concentrations of ozone. There are also some studies where exposure was by face mask or mouthpiece. The controlled human exposure studies reviewed by California are grouped into those that examined:

- the effect of one to four hour exposure on lung function (Table 11-3)
- airway responsiveness (Table 11-4)
- inflammatory effects measured in bronchoalveolar lavage fluid (Table 11-5)
- other studies of inflammatory and host defense effects (Table 11-6)
- pulmonary function effects with prolonged exposures to ozone (Table 11-7)
- airway hyperresponsiveness and inflammatory effects with multi-hour ozone exposures (Table 11-11)
- effects on patients with pulmonary or cardiovascular disease (Table 11-12)
- effects on patients with gender and hormonal differences (Table 11-13)
- influence of age on pulmonary function changes (Table 11-14)
- influence of ethnic, environmental and other factors on response to ozone (Table 11-15)
- pulmonary function effects with repeated exposures to ozone (Table 11-17)
- responses to mixtures of ozone and sulfur-containing pollutants (Table 11-18)
- responses to mixtures of ozone and nitrogen-containing pollutants (Table 11-19)
- responses to mixtures of ozone and peroxyacetylnitrate (Table 11-20)
- responses to mixtures of ozone and particulate matter (Table 11-21).

Most of the controlled human exposure studies were tests of lung function. These tests generally included measurement of:

- Forced vital capacity (FVC) - the total amount of air that can be exhaled or inspired
- Forced expired volume in one second (FEV_1) – the volume expired in the first second of maximal expiration after a maximal inspiration and is a useful measure of how quickly full lungs can be emptied. FEV_1 is considered the most reproducible measure of acute changes in large airway.
- $FEF_{25-75\%}$ - Average expired flow over the middle half of the FVC maneuver. It is considered an indirect measure of the caliber of smaller airways at lower lung volumes.

Tests other than lung function included examination of hyperactivity, inflammation, defense mechanisms, symptoms (e.g., coughing, pain on deep breath, etc.) and cardiovascular outcomes.

Many of the studies examined pulmonary function for a relatively short time period (1-4 hours). Table 11-3 of the Document describes the pulmonary responses following 1-4 hours of exposure for various concentrations of ozone. In the 31 studies reported in Table 11-3 of the Document, ozone exposures ranged from 0.08 to 0.75 ppm. Most of the later studies examined the effect of ozone on individuals performing moderate to heavy exercise, while the earlier studies examined the effect of ozone on individuals at rest. Nine of the studies listed in Table 11-3 included a concentration of 0.125 ppm or lower in their testing protocol. The length of time and the amount of exercise varied in the test protocols which of course affected the levels at which effects are seen. Of the nine studies which examined exposure at concentrations of 0.125 or lower, four found significant pulmonary function decrement at 0.12 ppm (McDonnell et al. 1983, McDonnell et al. 1985, McDonnell et al. 1993, Seal et al. 1993). Kulle et al. (1985) reported a threshold for response above 0.10 ppm but below 0.15 ppm. As indicated by the Document, the group mean decrements in pulmonary function at 0.12 ppm for the short (1-4 hours) exposures have been relatively small, 3-5% in FEV_1, but maximum individual decrements have been as much as 29% (Gong et al. 1986).

Statistically significant group mean decrements in FEV_1 have been found to occur at 6.6 to 8 hour ozone concentrations as low as 0.08 ppm. Similar to the decrements seen in the one to four hour exposures, the decrements for these longer time periods are rather small (2 to 8%). Again, however, there is considerable variability, and certain individuals experienced much higher decrements. For example, according to the Document, Folinsbee et al. (1991) reported that in a 6.6-hour study 26% of subjects at 0.08 ppm had FEV_1 decrements greater than 10%, and 10% had decrements greater than 30%. Horstman et al. (1990) reported an FEV_1 decrement of 25.9% in one individual exposed to 0.08 ppm. The group mean decrement at that exposure was only 6.6%, however.

Significantly increased symptoms of respiratory irritation have been reported, both in number and severity, at concentrations of 0.12 ppm with heavy exercise following 1-3 hour exposures. Such symptoms also include cough, shortness of breath, etc. Increased respiratory symptoms have also been observed at 6.6 hour exposures with moderate

exercise at concentrations as low as 0.08 ppm including cough, shortness of breath, and pain on deep inspiration (McDonnell et al. 1991).

Airway inflammation has been observed following 1 to 3 hour exposures of healthy adults to 0.20, 0.30, and 0.40 ppm ozone with heavy exercise. No studies have investigated airway inflammation after 1 to 3 hours at ozone concentrations lower than 0.20 ppm. Inflammation of airways has also been demonstrated at 0.08 and 0.10 ppm after 6.6 hours of exposure

KEY STUDIES

Particularly relevant to the proposed 1- and 8-hour standards are those studies which examined exposures close to the proposed standards and those studies with durations of exposure most relevant to the proposed standards (i.e., 1 hour and 8 hours). Four studies which deserve special mention are those of Gong et al. (1986), Horstman et al. (1990), McDonnell et al. (1991), and Adams (2002). These studies are of particular significance because of the low concentrations at which the volunteers were tested, and because, other than Gong et al., they recorded responses hour by hour. Gong et al. studied performance cyclists doing continuous exercise for one hour. Horstman et al. tested exercising volunteers at 0.0, 0.08, 0.10, and 0.12 ppm for 6.6 hours, McDonnell et al. tested exercising volunteers at 0.08 ppm for 6.6 hours, and Adams tested exercising volunteers for 0.04, 0.08, and 0.12 ppm for 6.6 hours.

Gong et al. (1986)

Gong et al. evaluated the effect of low concentrations of ozone on the exercise performance and pulmonary function of 17 (15 male, 2 female) top caliber endurance cyclists under conditions simulating competition at a temperature of 31º C at the UCLA Medical Center. Each subject underwent 3 randomly assigned exposures – 0.12 ppm O_3, 0.2 ppm O_3, and filtered air at approximately the same time each day for one hour. The study was double-blinded (neither the technicians administering the test nor the subjects inside the chambers were aware of the concentration). None of the subjects reported active asthma or the use of medications including bronchodilators. Each subject was asked to maintain submaximal exercise on a cycle ergometer for 60 minutes followed by maximal exercise (cycling at 75 rpm) until exhaustion. Maximal exercise was initiated either at the conclusion of the 60 minute submaximal period or when the subject complained about intense symptoms. Exercise was terminated when the subject was unable to continue cycling at 75 rpm despite encouragement. After a 3 minute cool-down period each subject underwent sequential measurements of exhaled gases and spirometry.

Exposure to 0.12 ppm ozone resulted in modest but significant post exercise decrements in FVC (7.6%, $P< 0.001$) and FEV_1 (5.6%, $p < 0.02$). (**Note**: The Document indicates that the decrements were significant at 0.2 but not at 0.12 ppm ozone. Gong et al. reported that they were significant at both exposures.) Exposure to ozone at 0.20 ppm produced large post exercise decrements ($p<0.001$) in FVC (19%), FEV_1 (22%), and Maximum Voluntary Ventilation (18%). The control group, exposed to filtered air

showed no significant decrement and actually registered a 4.1% increase in FEV_1 ($p < 0.01$). The number of symptoms and number of subjects reporting symptoms increased with increasing ozone concentration. The most frequent complaints were tightness and soreness of the chest and shortness of breath even during exposure to 0.12 ppm. The authors concluded that the threshold level for a significant ozone effect on lung function with heavy continuous exercise is below 0.12 ppm for most persons. The authors found an increase in airways responsiveness to histamine at 0.20 ppm.

Horstman et al. (1990)

Horstman et al. studied 22 healthy, nonsmoking male volunteers at EPA's Health Effects Research Laboratory in RTP, NC. Each subject was exposed to 0.0 (filtered air), 0.08, 0.10, and 0.12 ppm ozone on separate days. Exposures were separated by a week, and the exposure sequence was randomized. The study was double-blinded as to the concentration of ozone (i.e., neither subjects nor staff involved in conduct of the experiments were informed as to the concentration of ozone in the chamber). Subjects and staff were also not informed as to the presence of ozone in the chamber, but the authors claimed that it was not possible to blind the presence or absence of ozone because of the odor of O_3. Baseline lung functions were measured, and respiratory symptoms were evaluated before exposure. The subject entered the chamber and began exercising at a previously determined exercise intensity for six 50-minute periods each followed by a 10 minute evaluation period. After the third exercise period, the subject was given 35 minutes to eat lunch. Measurements included forced expiratory spirometry and symptom readings after each exercise period, airway resistance after the 3^{rd} and 6^{th} exercise periods, and a metacholine challenge (to measure airway reactivity) after the 6^{th} exercise period. Three variables were determined from previous studies to be the variables with the most potential for demonstration of O_3-induced changes - FEV_1, PDI, and PD_{100}. FEV_1 is described above. PDI is pain on deep inspiration; PD_{100} is the dose of metacholine that provokes a 100% increase in airway resistance. Except for PD_{100} post exposure-pre exposure differences at each of the O_3 concentrations were compared to postexposure-pre exposure differences in clean air using a nonparametric version of Williams' test for a randomized block design. For PD_{100}, only the postexposure values were compared using Williams' block design.

Significant decreases in mean FEV_1 were found at all ozone concentrations from pre- to post-exposure. The change in FEV_1 from pre- to post-exposure for 0.08 ppm ranged from +7.9% to -25.9%. The provocative dose of metacholine required to increase airway resistance by 100% at all three concentrations was also significantly reduced at all three concentrations. PDI was significantly increased at all three ozone concentrations when compared to clean air.

The decrements in FEV_1 were not statistically significant until 5.5 hours after the 0.08 ppm exposure began, 4.5 hours after the 0.10 ppm exposure began, and 3 hours after the 0.12 ppm exposure began. The range of decrements at each time period after exposure began was not provided by the authors. On the day after exposure, FEV_1 returned to within 1% of baseline FEV_1 measured before each exposure demonstrating that, at least

for the short term exposure to which subjects in this study were exposed, the results with respect to FEV_1 were reversible.

McDonnell et al. (1991)

McDonnell et al. studied 38 healthy, nonsmoking male volunteers at the EPA's Health Effects Research Laboratory in RTP, NC to ozone. The subjects were different than those studied in Horstman et al. described above. Twenty-eight of the subjects were exposed twice, once to clean air and once to 0.08 ppm. Each of the 10 additional subjects were exposed on three occasions to clean air, 0.08 ppm O_3, and 0.10 ppm O_3. Exposures were separated by at least two weeks. The protocol was similar to the Horstman et al. study described above with volunteers exercising for 50 minutes followed by 10 minutes of rest, spirometry, and symptom evaluation. The third exercise period was followed by a 35 minute lunch break. The measured variables were also similar. Statistical significance was evaluated using paired t-tests.

Significant decrements in FEV_1 and PC_{100} (a measure of airway resistance) were observed following exposure to 0.08 ppm O_3 compared with clean air. Other variables for which significant changes were observed for exposure to 0.08 ppm O_3 include FVC, FEF_{25-75}, PEF (peak expiratory flow), FEV_1/FVC, SR_{aw} (specific airway resistance), cough, and inspiratory difficulty. An exposure-response pattern was observed for the 10 subjects who also underwent exposure to 0.10 ppm ozone. The 0.10 ppm produced a stronger response in terms of FEV_1 decrement than for 0.08 ppm. For both the .08 ppm and 0.10 ppm exposures the FEV_1 decrement increased with duration of exposure. The decrement in FEV_1 as a function of exposure duration was curvilinear and very similar to that observed by Horstman et al. Neither the range of FEV_1 decrements nor the statistical significance of the FEV_1 decrement by time period was reported by the authors. Overall, however, the FEV_1 decrements by time period for the 0.08 ppm exposure appeared similar to those observed by Horstman et al. Also, similar to Horstman et al., the authors found a wide range of changes in FEV_1 from pre- to post-exposure for the 0.08 ppm exposure (+4.3% to -37.9%).

Adams (2002)

Adams exposed 30 healthy, nonsmoking young adults, 15 of each gender who had not lived in an area for 6 months where the State of California air quality standard for O_3 was exceeded (0.09 ppm for one hour) in the Human Exposure Laboratory at the University of California, Davis. Five exposure protocols were completed by each subject: (1) a 6.6 hour exposure in a chamber to 0.12 ppm O_3 with six 50-minute exercise periods at a ventilation rate of 20 $L/min/m^2$, (2) the same 6.6-hour protocol while exposed to filtered air, (3) the same as the first protocol except that a face mask was used rather than exposure in a chamber, (4) the same face mask protocol with exposure to 0.08 ppm, (5) the same face-mask exposure with exposure to 0.04 ppm. The protocol for the study was similar to that employed by Horstman et al. and McDonnell et al.

Post-exposure percent change in FEV_1 was not significantly different from that for the same face mask exposure (protocol 1 vs. protocol 3). Post exposure change in FEV_1 for protocol 4 (0.08 ppm) was significantly different from that for filtered air (protocol 2) and from protocol 5 (0.04 ppm O_3). Post-exposure percent change for FVC for all protocols closely paralleled those for FEV_1. The change in FEV_1 over time was plotted by hours since the start of exposure. The FEV_1 percent change from pre-exposure was significantly greater for protocols 1 and 3, and was significantly different from that for filtered air by the third hour. The percent FEV_1 change for protocol 4 (0.08 ppm O_3) was significantly different by the 5^{th} hour. Percent changes for protocol 5 (0.04 ppm ozone) did not differ from that for filtered air. The range of decrement in FEV_1 by time period was not provided by the author

Pain on deep inspiration (PDI) and total symptom score (symptoms included throat tickle, cough, shortness of breath, and pain on deep inspiration) were significantly greater for protocols 1 and 3 than for the other protocols. Total symptoms score, but not PDI, was greater for protocol 4 (0.08 ppm O_3) than for filtered air but not significantly different from protocol 5 (0.04 ppm O_3). Total symptoms score became significant at the third hour of exposure for the two 0.12 ppm O_3 exposures (protocols 1 and 3). The total symptom score for protocol 4 (0.08 ppm O_3) did not become statistically significant until the 6^{th} hour. Total symptom score did not change significantly during either protocol 2 (filtered air) or protocol 5 (0.04 ppm O_3).

DISCUSSION

None of the controlled human studies has observed effects at the current one-hour standard of 0.09 ppm O_3 or the proposed 8-hour standard of 0.07 ppm O_3. The lowest O_3 concentration at which statistically significant effects were reported in a controlled human exposure in a time period approaching one hour is 0.12 ppm. The lowest concentration at which statistically significant effects have been reported in a controlled human study approaching eight hours is 0.08 ppm O_3.

The chamber studies focus for the most part on decrements in lung function in response to ozone exposure, particularly FEV_1 and other measures of lung function. With regard to such measures of physiological impact, the American Thoracic Society (ATS) (2000) concluded that small, transient loss of lung function should not automatically be considered adverse. It was demonstrated by Horstman et al. (1990) that the decrement in FEV_1 following 6.6 hours of exposure to 0.08 - 0.12 ppm ozone is reversible, and certainly the decrements in FEV_1 could be considered small (approximately 5%). The ATS (2000) went on to state that "in drawing the distinction between adverse and nonadverse reversible effects, this committee recommended that reversible loss of lung function in combination with the presence of symptoms should be considered adverse." They further indicated, however, that "characterizing the degree of symptomatology associated with diminished quality of life is an appropriate focus for research and a topic that could be investigated using new approaches for assessing quality of life." The Document did not address this important issue as it relates to the setting of a standard or the need for research in characterizing the degree of symptomatology.

Group mean FEV_1 decrements at 0.12 ppm after 1-3 hours of exposure are modest (3-5%), but as has been noted, individual decrements can be quite large (as much as 29%). The group mean decrements at 0.08 ppm following 6.6 hours of exposure are again modest (2-8%) but again the individual decrements have been found to be as much as 25%. Comparisons of the FEV_1 before and after ozone exposure generally did not consider this extreme variability of response (i.e., distributions were assumed to be normal, which is likely a flawed assumption) and accordingly group means were compared. Indeed, in the Horstman et al. study, the authors used a nonparametric approach in their analysis since they found a significant lack of fit to a normal distribution. For their comparison of the time points at which significant decrements became significant, however, Horstman et al. assumed normality and used paired t-tests. These important statistical issues were not addressed in the Document.

Few, if any, studies examined the response by individual for different exposures. For example, in the Horstman et al. study, the FEV_1 decrement was monotonic with respect to exposure concentration for only 7 of the 22 individuals in the study, and for two of these the FEV_1 actually increased with increasing exposure to ozone. The Document provided no discussion of this apparent lack of dose response by the majority of the subjects. As Horstman et al. indicate, the subjects in the study likely had knowledge they were being exposed to ozone because of the odor but were blinded as to the concentration. While ozone appears to have an effect in each of the exposure groups, the lack of a monotonic response across ozone concentrations for many of the subjects suggests that the smell of ozone could have produced a response bias. A number of the other studies on which the Document is based claimed that the subjects were blinded as to their exposure. Again, however, that may be true with regard to the concentration of ozone but not to the presence of ozone and thus response bias may be an issue in these studies as well.

Because the proposed California standards are for one and eight hours, the Document should place stress on the studies which focus on these periods of time. Few, if any, studies actually exposed people for as long as eight hours, but there were several studies that exposed individuals for 6.6 hours, and effects were reported at those concentrations (subject to the limitations discussed above). Twelve of the 31 studies in Table 11-3 of the Document (One to Four Hour Exposures to Ozone-Pulmonary Function) examined one hour exposures; of these, however, only three examined exposures less than 0.2 ppm ozone. Of those three, two examined exposures as low as 0.12 ppm ozone, one of which found a significant decrease (Gong et al. 1986) and one of which did not (Schelegle and Adams 1986). Both studies were conducted on highly trained endurance athletes. The Gong et al. protocol was run at 31º C (about 88º F) and 35% relative humidity whereas the Schelegle and Adams study maintained temperatures at 23-26º C (about 73-79º F) and 45-60 % relative humidity suggesting that there could be climate and seasonal differences with respect to ozone. This issue was not addressed in any detail in the Document.

The effects observed at 0.08 ppm after 6.6 hours or at 0.12 ppm after 1 hour were in subjects engaged in moderate to heavy exercise. In fact, the effects observed by Gong et al. at 0.12 ppm followed an hour of extremely intense exercise in which the subjects

(world class cyclists) were pushed to their absolute maximum exercise level in a temperature of 88° F and 35% relative humidity. The intensity of exercise and the conditions in many of the studies are well beyond what most of the general population would experience. Whether the effects seen under the conditions employed in the various controlled human exposure studies are applicable to a general population standard has not been considered by the Document.

In summary, California has accepted at face value the controlled human exposure studies described in the Document without the rigorous examination that it should have invested.

EPIDEMIOLOGY

Some supporting evidence for the effects seen in the controlled human exposure studies is provided by epidemiological studies. Because the commonly used metrics of exposure (1 hour peak exposure, 8 hour average exposure, 24 hour average exposure) are highly correlated, epidemiological studies provide only limited guidance regarding the form or the level of the standard. However, epidemiological studies do provide evidence of association between ambient concentrations of ozone at or below current air pollution standards and adverse effects on human health. The strength of the epidemiological evidence depends upon the specific study design. The best evidence of an ozone association with adverse effects on human health comes from studies of cohorts of individuals followed over time with longitudinal monitoring of either pulmonary function or of respiratory symptoms. Weaker evidence is provided by time-series studies exploring the association between ambient concentrations of ozone in a city and its association with the numbers of emergency room visits for asthma, hospital admissions for respiratory and cardiovascular causes, or non-accidental mortality on the same or subsequent days. Finally, the large cohort studies investigating the association between air pollution and mortality provide little evidence of an association between ozone and mortality. We discuss the evidence from each of these three classes of study below.

LONGITUDINAL STUDIES

A number of studies examining the associations between components of air pollution and respiratory function, lung growth and respiratory symptoms have been carried out within the last few years. While, taken together, these studies provide some evidence of associations between air pollution and the respiratory end points mentioned above, the evidence regarding specific components, including ozone, is mixed. Additionally there are inherent limitations to each of these studies so that the interpretation of results is not straight forward. We review some of the more recent studies, several of which were referenced in the Document. A number of recent important studies have been conducted in California by groups at the University of California at Berkeley and the University of Southern California (USC). Other studies have been conducted in the eastern United States and in Europe. While these studies are often referred to as cohort studies, they are actually semi-ecologic; whereas outcomes are measured on an individual basis, exposure to air pollution is only known on a group level from central monitors.

Among the largest and best conducted studies in the U.S. is the recent study by Gent et al. (2003). While this study was meticulously conducted and analyzed by a group of highly competent investigators, some of the inherent limitations of this study should be recognized. The study investigated the association of ambient fine particles and generally low levels of ambient ozone with respiratory symptoms in children with asthma. Two hundred and seventy one children below the age of 12 in southern New England participated in the study. Daily respiratory symptoms and medication use were prospectively recorded over the six-month period, April 1, 2001 to September 30, 2001. The authors concluded that asthmatic students using maintenance medication (the children with more severe asthma) were particularly susceptible to developing respiratory symptoms in response to exposure to high levels of ambient ozone. No such association was found with fine particles in joint pollutant models with ozone. One of the limitations of this study is that ozone and fine particles were the only pollutants considered. In particular NO_2, which has been found to be associated with pulmonary functions and respiratory symptoms in other studies, was not included in the analyses. Also, temperature and relative humidity could be confounders of the ozone association. Although limited control for temperature is reported in the paper, relative humidity does not appear to have been controlled. As the authors point out, they have no information on potential confounders or effect modifiers such as race and socioeconomic status (SES). The study design allows each individual to act as his/her own control and thus confounding by these factors is probably not an issue; however possible effect modification remains an issue. Finally, it is puzzling that, while the results clearly suggest a dose related increase in respiratory symptoms, there is little indication of increased use of bronchodilators, which would have been expected.

Other studies of respiratory symptoms have reported no associations with ozone. For example, a prospective study of air pollution and bronchitic symptoms in children with asthma by McConnell et al. (2003) in southern California reported the strongest associations of bronchitic symptoms with organic carbon and NO_2. In single pollutant models the risk associated with ozone was modestly elevated and border-line significant. However, in joint pollutant models with organic carbon or NO_2, the ozone association decreased and was no longer significant. In an earlier cross-sectional study (Peters et al., 1999) examined the association between various indices of air pollution and respiratory morbidity among 3676 southern California school children. They found associations of wheeze both with acid and NO_2, but not with ozone.

Among the studies conducted in California, one of the most interesting is the cohort study of asthma among exercising children (McConnell et al., 2002). The investigators followed a group of 3535 school children with no history of asthma in 12 southern California communities, 6 with high ozone concentrations and 6 with low ozone concentrations. The investigators report that in communities with high ozone concentrations, the relative risk of developing asthma in children playing 3 or more sports was 3.3 compared with children playing no sports. The investigators, interpreting their results say, “Incidence of asthma is associated with heavy exercise in communities with high concentrations of ozone, thus, air pollution and outdoor exercise could contribute to the development of asthma in children.” This study has a number of limitations that

should be noted. First, because ambient pollutant concentrations are highly correlated, a number of pollutants must have been high in the high ozone communities. We might note here that in their conclusions the authors appear to attribute the asthma in children to high air pollution rather than ozone although this is not clearly stated in the paper. While the authors have information on other pollutants it is not clear whether any co-pollutants models were used in the analyses. Similarly it is not clear how potential confounders listed in Table 1 of the McConnell et al. paper were adjusted in the analyses. Table 5, which presents the results of playing team sports in low and high ozone communities, shows no apparent dose-response relationship with respect to the number of team sports played. Table 4 shows the same pattern of relationships for PM as Table 5 does for ozone suggesting also that the effect may be an air pollution effect rather than an ozone effect. Finally, a surprising finding is that the overall risk of developing asthma is not higher in the six high pollution communities. In fact the authors say, "Communities with high NO_2 and associated pollutants, and *communities with high ozone$_{10\text{-}18}$ or daily maximum ozone were associated with a decreased risk of asthma*; these associations were significant ($p<0.05$) only for daily maximum ozone." (Emphasis added). Overall, this study provides little evidence of an air pollution, let alone an ozone, effect in the development of asthma.

Several longitudinal studies of the association between air pollution and pulmonary function and lung growth have appeared in the last few years. The evidence regarding the role of ozone has been mixed. For example, a recent study by Gauderman et al. (2000) among southern California school children concluded, "…significant negative effects on lung function growth in children occur at current ambient concentrations of particles, NO_2, and inorganic acid vapor." These investigators found no affect of ozone. In contrast, in another southern California cohort, the same group of investigators (Gauderman et al., 2002) reported an association between ozone exposure and reduced growth in peak flow rate, but not other measures of pulmonary function, such as FEV_1, or midexpiratory flow. These were associated with other components of the air pollution mixture, such as acid and NO_2.

In a large European study, Frischer et al. (1999) followed a cohort of 1150 children from 8 distinct locations with different pollution profiles for 3 years (1994, 1995 and 1996) to investigate the long-term effects of ambient ozone on lung function growth. On each of the study participants, the investigators measured lung function twice a year, once between March and May and again between September and November. The period between the first and second examinations was called summer that between the second examination and the first examination of the subsequent year, winter. The authors concluded, "Long term ambient ozone exposure might negatively influence lung function growth." A weakness of the study was that no multiple pollutant models were considered and no SES adjustments were apparently made. Although the investigators reported an association between ozone and lung function growth, there was apparently no effect modification by asthma or atopy status, i.e., asthmatic and atopic children were not at increased risk. Finally, if the decrease in lung function growth were irreversible one would expect a much decreased lung function in the areas of high ozone concentration than in areas with the lowest ozone concentrations. Unfortunately, the investigators did not report whether this was true. In a commentary on this paper, Tager (1999) concluded

that the investigators had not made their case for ozone being the pollutant responsible for the observed declines in lung function.

In summary, the longitudinal epidemiological studies do not paint a consistent picture of the association between individual components of air pollution and either decreased pulmonary function or respiratory symptoms. Furthermore, there is no clear indication from these studies that asthmatic individuals are at higher risk.

TIME SERIES STUDIES

The great majority of epidemiological studies of air pollution are time-series studies in which daily counts of events, such as hospital admissions or deaths, in a geographic area are regressed against levels of air pollution as measured at central monitoring stations in that area. In the time-series study, inferences regarding the association of air pollution with adverse health effects depend upon relating fluctuations in daily counts of the health effect of interest to levels of air pollution on the same or previous days. This type of study is ecologic in that both exposure to air pollution and the outcome of interest are measured on the population level. In 2002, the most commonly used software package (S-plus) for Generalized Additive Model (GAM) analyses of time-series studies was found to yield misleading results when used with the default convergence criteria, casting doubt on the results of most time-series studies of air pollution. Most time series studies of ozone have not been reanalyzed following the discovery of the software problem. Reanalyses of time series studies have focused mainly on PM. Thus, the results of most time series studies of ozone cannot be trusted. As with PM it is likely that reanalyses of the ozone studies using more stringent convergence criteria would lead to smaller effects estimates and reduced significance of the ozone associations. Even more important, the reanalyses prompted by the software convergence problem once again brought to the fore a number of issues, such as the proper control of weather and temporal trends in time-series analyses, which had been considered settled. These issues are far more serious than the convergence problems that led to their resurfacing. These problems, and others discussed below, in the interpretation of time series studies of ozone should be explicitly acknowledged and discussed in the Document. While the Document states quite clearly that time series analyses provide only weak support for an ozone standard, it does not discuss the substantial problems with currently available time series analyses. We believe that some discussion of these issues is in order to put the results of such analyses into perspective. While many of the considerations discussed below apply to other study designs, they are particularly relevant to time series studies of air pollution epidemiology because of the tiny risks being estimated and the difficulty of controlling confounders such as weather and temporal trends.

With respect to confounding in time-series studies there are three major issues that must be addressed. First, can adequate adjustments be made for temporal trends in the health effect of interest due, for example, to temporal trends in the structure of the population or to episodic viral infections? Second, can the association of pollutants be teased apart from the effects of climate and weather? Third, can adequate statistical adjustments be made so

that the association of ozone with adverse effects on human health can be teased apart from the associations of other criteria pollutants with adverse effects on human health?

The revised analyses necessitated by the S-plus problems clearly indicate that methods used for controlling temporal trends and weather can have profound effects on the results of time-series analyses of air pollution data, as has been noted by the HEI Expert Panel (2003). To make matters even more difficult, there appears to be no objective statistical test to determine whether these factors have been adequately controlled in any analysis. The HEI Expert Panel for the reanalyses stated, "Ritov and Bickel (1990) have shown, however, that for any continuous variable, no strictly data-based (i.e., statistical) method can exist by which to choose a sufficient number of degrees of freedom to insure that the amount of residual confounding due to that variable is small. This means that no matter what statistical method one uses to select the degrees of freedom, it is always logically possible that even if the true effect of pollution is null, the estimated effect is far from null due to confounding bias." In other words, it is impossible to adjust temporal trends without accurate information from external sources regarding the appropriate degrees of freedom to be used. Such information simply does not exist. No conclusions can be drawn from time-series studies unless the results are robust to extensive sensitivity analyses. Most time-series studies in the literature have undertaken only limited sensitivity analyses, if at all. This is an issue that transcends the convergence problem and applies to any time series study of air pollution whether or not GAM was used for analyses. In particular, time series studies done before GAM analyses came into vogue, some of which are referenced in the Document, are not immune to this problem.

Issues of confounding of air pollutant associations by temporal trends, weather, and copollutants can be more generally discussed under the rubric of model choice. It is clear that the uncertainties in the estimates of pollutant effects are almost certainly understated by consideration of the statistical uncertainty computed under the fitted model alone. Much more uncertainty derives from the lack of information regarding the choice of appropriate models for adjusting confounding by other covariates, and the choice of appropriate lag structures. As Lumley and Sheppard (2003) point out, "Estimation of very weak associations in the presence of measurement error and strong confounding is inherently challenging. In this situation, prudent epidemiologists should recognize that residual bias can dominate their results. Because the possible mechanisms of action and their latencies are uncertain, the biologically correct models are unknown. This model selection problem is exacerbated by the common practice of screening multiple analyses and then selectively reporting only a few important results."

More recently others have expressed similar concerns in the peer-reviewed literature. In a recent publication, which uses the method of Bayesian Model Averaging (BMA), Koop and Tole (2004) say,

> "The main empirical finding of the paper is that standard deviations for air pollution-mortality impacts become very large when model uncertainty is incorporated into the analysis. Indeed they become so large as to question

the plausibility of the previously measured links between air pollution and mortality."

BMA is not really a new idea. In the area of air pollution epidemiology it has been used by Clyde (2000) and colleagues to investigate the influence of model choice on estimated air pollution effects. It might be argued that BMA is a 'shotgun' approach to analyses of epidemiological data. However, in the absence of biological information on appropriate lag structures and covariate adjustments it is most definitely one approach to investigating the uncertainty associated with model choice. If nothing else, it has the virtue of being an objective arbiter of model choice.

In summary, because of the tiny risks being estimated, the difficulties of controlling weather and temporal trends and in the choice of the appropriate lag structure, the results of currently available time series analyses of air pollution cannot be accepted with any degree of confidence. The Document properly recognizes the limited role these studies play in setting an ozone standard. Even if one were to take the results of existing time series studies at face value, these results are mixed with some studies suggesting a role for ozone in hospital admissions and mortality and others not. Among those studies that find a positive association with ozone many report an association in summer but not in winter. One study, the National Mortality Morbidity and Air Pollution Study (NMMAPS), is of particular note because it was reanalyzed after the S-plus convergence problem was discovered and also because it is probably the most comprehensive time series analysis of ozone undertaken. We summarize the results of this study and point out its significant limitations.

NMMAPS was an ambitious effort, funded by HEI and carried out by investigators at Johns Hopkins and Harvard Universities, to conduct comprehensive time-series analyses using a unified approach, of the association between PM_{10} and mortality in the 90 largest metropolitan areas in the US and between PM_{10} and hospital admissions in a subset of these areas. The gases, including ozone, were considered as possible confounders of the PM effect. The focus of NMMAPS was PM and as a consequence, NMMAPS did not systematically explore the association between any of the gases and either mortality or hospital admissions. In the hospital admissions part of the study, no attempt was made to estimate the association between the gases and hospital admissions. In the mortality part of the study, the individual estimates of risk for PM and each of the gases in each city were combined in a second step using a Bayesian procedure to arrive at a single 'mean' estimate of risk. This approach ensured that identical models and lag structures were used for analyses and that confounding factors were treated in the same way. This approach raises its own problems, however. For example, is it appropriate to treat temperature and relative humidity in the same way in cities as disparate as Los Angeles and Chicago? After the S-plus convergence problem was discovered the investigators reanalyzed the data using both more stringent convergence criteria and alternative modeling approaches using Generalized Linear Models (GLM). After the revised analyses, they reported that the mean effect was positive, although substantially smaller than the original estimate, and statistically significant. However, the PM coefficients in individual cities and their level of significance were substantially reduced with the more stringent convergence

criteria and even further attenuated with the use of GLM instead of GAM. In these revised analyses in the 90 cities a substantial number of estimated effects were either negative or close to zero and only two were positive and statistically significant. We do not believe that such heterogeneous results should be combined using a Bayes (or any other) procedure.

With respect to the association between ozone and mortality, the NMMAPS investigators concluded, "Ozone was associated with total mortality in the summer months. In our judgment, the new sources of uncertainty arising from model choice lead to quantitative changes in estimates without qualitative implications." The investigators do not comment, however, on the strong negative association between ozone and mortality in the winter months. Moreover, the issue of the influence of model choice on the ozone association was dismissed in the one sentence quoted above. The investigators tried neither a formal procedure, such as BMA, nor extensive sensitivity analyses to see whether and to what extent the ozone associations were affected by model choice. Detailed city-specific results for each of the pollutants have been posted on the Johns Hopkins web site. For ozone, however, it is not clear whether the results are for full-year, summer or winter analyses. Nevertheless, the posted results indicate substantial heterogeneity from city to city suggesting that a single mean estimate of risk is inappropriate.

The strength of NMMAPS is at the same time its weakness. While NMMAPS is the first attempt to look at the 90 largest metropolitan areas in the United States using a unified approach to analyses, it could also be argued that using identical control for weather and temporal trends across the entire country is inappropriate. In any case, the new information regarding the sensitivity of results to model choice discussed above makes it clear that NMMAPS cannot provide the kind of insights that it was intended to provide. Given these limitations of NMMAPS, it is difficult to interpret the positive association between ozone and total mortality in this study.

In summary the time series studies provide at best weak evidence in support of associations between ambient ozone and various adverse health effects in human populations.

COHORT STUDIES

A few large cohort studies of the association between air pollution and mortality have been performed. In reality, these are semi ecologic studies because the exposure of interest, air pollution, is measured only at central monitors. The Document quite properly acknowledges that these provide little support for an ozone standard. However, the Document does say that the 2002 study by Pope et al. reports a positive association between ozone and mortality, which is almost significant. We feel that it is important to put this isolated result into context. The first cohort study to be undertaken, the Harvard Six Cities study, found little evidence of an association between exposure to ambient ozone and mortality. In fact, of the pollutants examined only ozone showed no evidence of association. The first ACS II study by Pope et al. did not examine the association

between any of the gases and mortality. However, a reanalysis of the same data by Krewski et al. found no evidence of association between ozone and mortality; these authors found the strongest association with SO_2. The 2002 paper by Pope et al. referred to above is an updated analysis with several more years of follow up. This paper reports, in addition to the almost significant association with ozone, strong associations with fine particles and SO_2. However, surprisingly, the paper presented no joint pollutant analyses and thus it is not clear which pollutant is most strongly associated with mortality or whether the reported ozone association is robust to the inclusion of other pollutants, particularly SO_2.

The Washington University/EPRI veterans study (Lipfert et al., 2000) is another large cohort study of air pollution and all-cause mortality. The cohort consists of approximately 50,000 U.S. veterans who were diagnosed with hypertension in the mid 1970s. Among the pollutants, the strongest mortality associations were seen with NO_2 and peak ozone. Of these two pollutants the authors reported that ozone showed the stronger association with mortality, although there was an indication of a threshold at about 0.14 ppm for ozone effects.

Contemporary examples indicate that confounding can be very difficult to control even in the most carefully conducted observational epidemiological studies. This problem of residual confounding is particularly acute when the risks that are being estimated are extremely small, as is the case in epidemiological studies of air pollution. The case of post-menopausal hormone replacement therapy (HRT) provides a dramatic example. Based largely on observational epidemiology studies bolstered by 'biological plausibility' HRT became one of the most prescribed therapies in the United States, "with a highly diversified portfolio of presumed benefits for post-menopausal women" (Herrington & Howard, 2003). Recent randomized trials (a randomized trial is the only way to assure that confounding is adequately addressed) of one form of HRT, combined estrogen and progesterone therapy, showed that this therapy does not slow the progression of cardiovascular disease, which was one of the important presumed benefits of HRT.

Changing smoking habits and changing life-style factors are two strong candidates for confounders in cohort studies of air pollution and mortality. We know that there have been profound changes in life-style and smoking habits over the period of this study. People generally are eating better, exercising more, and smoking less. These life-style changes are more likely to be adopted by the more affluent, better educated communities, which are also exposed to less air pollution. Thus, the association between air pollution and mortality may simply reflect the impact of changing life-style factors, including changes in smoking habits, on mortality. In particular, smoking is such a strong risk factor for mortality that controlling changing habits well enough to assure absence of residual confounding would be extremely difficult.

In summary, the current cohort studies of air pollution and mortality provide little evidence of an association between ozone and mortality. Because of the real possibility of residual confounding by life style factors in these studies any reported air pollution association must be interpreted with caution.

DISCUSSION

While making no attempt to be comprehensive, the California Document derives, in general, reasonable conclusions from the review of the epidemiological literature. We do not take issue with its conclusions. However, in its discussion of the epidemiology it often fails to provide the appropriate context and perspective. For example, in its discussion of time series studies, while it recognizes, implicitly if not explicitly, the issues with interpretation of GAM analyses with the less stringent convergence criteria, it makes no reference to the whole host of model selection issues that have arisen. While it is true that most of these issues have arisen specifically with respect to PM, it is clear that they are equally relevant to ozone. With respect to the long term studies of air pollution and mortality, the document should acknowledge the real possibility of residual confounding in these studies and the fact that most of these studies showed no association between ozone and mortality. Finally, we feel that the document should acknowledge the extreme difficulty of eliminating residual confounding in observational epidemiological investigations of very small risks.

ANIMAL TOXICOLOGY STUDIES

The Document briefly discusses a number of animal studies to provide a biologically plausible basis for the view that individuals exposed to ozone over a lifetime may experience chronic lung injury. As with the controlled human studies, we focus in this review on the studies that are cited in the Executive Summary of the Document.

Last et al. 1994

Male and female Fischer-344/N rats were exposed to 0, 0.12, 0.5, or 1.0 ppm ozone, 6 hours per day, 5 days per week, for 20 months (Last et al. 1994). Each exposed group consisted of 6 rats, except for the groups exposed to 0.12 ppm, which consisted of 3 rats. Collagen deposition in lung tissue was examined to determine whether exposure caused pulmonary fibrosis. Excess collagen deposition occurred in both male and female rats exposed to 0.5 or 1.0 ppm of ozone. No significant changes occurred in the lungs of any of the rats exposed to 0.12 ppm. The authors conclude, however, that the number of animals in this group was much too small to conclude that this was a no-observable-adverse-effect-level.

Reiser et al. (1987)

Two groups of juvenile cynomolgus monkeys were exposed to 0.61 ppm of ozone 8 hours per day for 1 year (Reiser et al. 1987). One group was killed immediately after cessation of exposure while the other group was killed after breathing filtered air for an additional 6 months. In the group killed immediately after exposure to ozone, changes in collagen cross linking were characteristic of those seen in lung tissue in the acute stage of pulmonary fibrosis. In the group that was killed six months after cessation of exposure, abnormal collagen synthesized during the period of exposure was irreversibly deposited

on the lung, although collagen synthesis at the time the animals were killed was normal. The authors concluded that long-term exposure to "relatively low levels" of ozone may cause irreversible effects in lung structure.

Catalano et al. (1995)

The Health Effects Institute (HEI), in collaboration with the NCI, funded eight studies to investigate rats exposed to 0, 0.12, 0.5, or 1.0 ppm ozone for 6 hours per day, 5 days per week for 20 months. These rats developed changes in their respiratory systems that were characteristic of chronic respiratory diseases. Catalano et al. (1995) used a statistical method, polish analysis, to determine whether grouped multiple endpoints from these various studies (centriacinar fibrosis, airway disease, and chronic rhinitis) were associated with ozone exposure. Although a trend towards increased response with increased ozone exposure was found for all three endpoints, only the trend for chronic rhinitis was significant. In addition, rats exposed to 0.5 or 1.0 ppm ozone had a statistically significant increase in chronic rhinitis over the control rats. No such statistically significant increase was found for the other two endpoints.

Pinkerton et al. (1995)

Pinkerton et al. (1995) studied the effects of exposure to ozone on the lungs of rats by performing morphometric, histochemical, and enzymatic analyses of selected airway paths of the tracheobronchial tree. Male and female F344/N rats were exposed to 0, 0.12, 0.5, or 1.0 ppm ozone for 6 hours per day, 5 days per week for 20 months. Significant alterations were found in stored secretory product in the trachea and bronchi; the bronchiolar epithelium was extended into the pulmonary acini of exposed rats; and elevated levels of antioxidant enzymes were found. The authors concluded that the effects of long-term exposure to ozone are dose-dependent and site-specific along the tracheobronchial tree and pulmonary acini of the lungs.

Pinkerton et al. (1998)

Since a number of earlier studies of the effects of ozone on rat lungs used only one time point, at 20 months, Pinkerton et al. (1998) studied the effects of a 3-month exposure. Forty-two F344/M rats were exposed to 0, 0.12, or 1.0 ppm ozone under identical conditions as the previously reported studies. Significant increases were found in the volume density of nonciliated epithelial cells lining the trachea and bronchi as well as bronchioles in the cranial region. Remodeling of the centriacinar region was significant after exposure to 1.0 ppm ozone but not to 0.12 ppm. These results were also obtained after the 20 months exposure. The authors concluded that long-term exposure, and not the effects of aging, leads to significant alterations in the lining of the airways and centriacinar region of the lung.

Szarek et al. (1995)

Szarek et al. (1995) investigated whether ozone alters the contractile responses of small bronchi in rats. Male and female Fischer 344 rats were exposed to 0, 0.12, 0.5, or 1.0 ppm of ozone for 6 hours per day, 5 days per week for 20 months. Ozone exposure was without statistically significant effect on maximum tension development or effective dose in response to pharmacological stimuli or electrical field stimulation. A significant increase in smooth muscle area in small bronchi occurred after 0.5 ppm of ozone. At 1.0 ppm, the effect of ozone on smooth muscle area did not attain statistical significance. After stress data were normalized to smooth muscle area, maximum responses of the small bronchi from male rats was found to be significantly reduced after 0.12 and 0.5 ppm ozone. A similar trend was observed in airways from the female rats but was not statistically significant. The results suggested to the authors that an increase in airway responsiveness associated with acute ozone exposure does not persist during chronic exposure. The authors suggested that smooth muscle cell function was compromised by chronic exposure but are uncertain of the relevance of these findings to humans.

Harkema et al. (1993)

Harkema et al. (1993) studied the effects of ozone on the surface epithelium lining respiratory bronchioles and on the bronchiolar interstitium in bonnet monkeys. Ten male and female Bonnet monkeys were divided into two groups exposed to either 0.15 ppm ozone for 6 or 90 days; six monkeys were exposed to 0.3 ppm for 90 days; and 5 monkeys composed a control group exposed to filtered air. All animals were exposed 8 hours per day. There were no significant differences in epithelial thickness or cell numbers among ozone-exposed groups. There was an increase in the exposed groups over the controls in the number of cuboidal cells, the thickness of the surface epithelium, and the number of cuboidal epithelial cells per surface area of basal lamina. The authors concluded that the exposure to low ambient concentrations of ozone induces pulmonary lesions in primates.

Tyler et al. (1988)

To compare the effects of seasonal and daily cycles of ozone exposure, Tyler et al. (1988) divided 18 male *Macaca fascicularis* monkeys into three groups: one group was exposed daily to 0.25 ppm ozone; the second group was exposed to 0.25 ppm ozone every other month; and the third group was exposed only to filtered air. Each group was exposed for 18 months. All the continually exposed monkeys were found to have respiratory bronchiolitis with significant increases in related morphometric parameters. The monkeys in the second group were found to have larger biochemical and physiological alterations and equivalent morphometric changes as those exposed daily in group 1. The authors concluded that the long term effects of ozone exposure, which has a seasonal occurrence, may depend more on the sequence of polluted and clean air than on the total number of days of pollution.

Schelegle et al. (2003)

Schelegle et al. (2003) investigated the effect of ozone exposure on monkeys that were sensitized to allergens. Twenty-four young rhesus monkeys were divided equally into four groups: two of which were sensitized to house dust mite allergen (HDMA) and two of which were not. Half of the sensitized monkeys were exposed to HDMA and the others were exposed to HDMA and ozone. Half of the non-sensitized monkeys were exposed to filtered air and the other half was exposed to ozone. Each of the exposed groups was subjected to 11 incidents, each incident consisting of 5 days of exposure followed by 9 days of filtered air. Ozone was delivered for 8 hours per day at 0.5 ppm. The airways of non-sensitized animals were only mildly affected by the ozone exposure. Sensitized monkeys exposed to HDMA also had mild airway effects except for a marked increase in the content of eosophinils in the proximal airway and terminal bronchiole. Sensitized monkeys exposed to HDMA and ozone, however, showed a marked increase in parameters of allergy including serum IgE, serum histamine, and airways eosinophilia, as well as alterations in airway structure and content. These results suggested to the authors that ozone can amplify the allergic and structural modeling of HDMA sensitization and inhalation.

Larson et al. (2004)

Further work on these lines was done by Larson et al. (2004) who investigated the effect of ozone on the distribution of airway nerves in atopic infant rhesus monkeys. Small conducting airways obtained from the monkeys studied by Schelegle et al. (2003) were examined to determine whether the postnatal development of the epithelial neural components within these airways were impacted by the repeated exposure to HDMA, ozone, or the combination. The neural distribution and density of nerve fibers located within the epithelial compartment of airways were compared using immuno-histochemistry for the four groups. The authors concluded that repeated cycles of acute injury and repair associated with episodic ozone and allergen exposure alter the normal development of neural innervation of the epithelial compartment and the appearance of a new population of undefined cells within epithelium.

DISCUSSION

The animal toxicology studies show that adverse effects on the lungs of rats and primates can result from controlled exposure to ozone. However, the evidence that adverse effects occur at ambient ozone concentrations is weak. Although Pinkerton et al. (1995) found changes in the ventilatory units of animals exposed to 0.12 ppm, this alteration was significant only in male animals. A later study by Pinkerton et al. (1998) showed no significant remodeling of the lungs following exposure to 0.12 ppm for either 3 or 20 months. Szarek et al. (1995) found significant effect of ozone on contractile response in small bronchi at 0.12 ppm, but only after normalizing the response with respect to smooth muscle area. In other studies, collagen deposition, chronic rhinitis, and airway remodeling, and pulmonary lesions occurred, but at levels at or exceeding 0.3 ppm; in each of these studies there was a lower exposure level at which no effects were observed.

Given the uncertainties associated with extrapolating from one species to another and from high to low doses, the cited studies are not useful for setting a quantitative standard to protect humans from the adverse effects of ozone exposure. They do, however, serve the purpose of illustrating that individuals exposed to high levels of ozone over a lifetime may be affected.

RESPONSE OF SENSITIVE GROUPS

Controlled human exposure studies have examined the effects of ozone on asthmatics, children, and people with allergies. There is suggestive evidence that asthmatics are more sensitive to the effects of an allergen following exposure to ozone. For example, Molfino et al. (1991) found that 7 subjects exposed to 0.12 ppm ozone for an hour followed by ragweed or grass allergen had a stronger response than when they were exposed to filtered air and then the allergen. Other studies found similar responses but at higher concentrations of ozone. Persons with allergies were also found to respond more strongly to allergens after exposure to ozone than after exposure to filtered air. There is little or no evidence that children responded more strongly to ozone than did adults in controlled human exposure studies. In fact, children reported fewer symptoms than did adults at similar ozone concentrations (but in different studies).

There is little epidemiological information on whether children, asthmatics and individuals with allergies are more susceptible than the general population to the potential adverse effects of ozone exposure. The few studies that address the issue have yielded mixed results. Thus, the study by Gent et al. (2003) reports stronger associations of ozone exposure with severe asthmatics (those on maintenance medications) than on mild asthmatics (those not on maintenance medications). By extension, it is plausible that asthmatics, in general, are more susceptible to ozone exposure than non-asthmatics. By contrast the study by Frischer et al. (1999) found no evidence that the association of ozone with decreased lung growth was modified by atopic status or asthma, i.e., there was no evidence that asthmatics and atopic individuals were at increased risk.

SUMMARY

The primary support for an ozone standard derives from the chamber studies, which focus primarily on decrements in lung function in response to ozone exposure. The decrements are relatively small, and they are reversible. Although symptoms have been observed following ozone exposure, there is some question as to whether such symptoms should be considered adverse. The epidemiological studies provide some additional evidence that ozone could have adverse effects on human health at levels at or below the current standards, but the evidence is ambiguous. Animal studies provide evidence for the biological plausibility of adverse effects resulting from ozone exposure, but these studies are not relevant to the setting of a quantitative standard.

The controlled human exposure studies are the primary basis of the California one- and eight-hour standards for ozone because such studies are considered free of potentially confounding factors such as particulate matter, heat, etc., and because exposure can be

measured precisely. Furthermore, exposures in the controlled human exposure studies are lower than those in the animal studies and closer to the proposed standards. Both the epidemiology and animal studies provide support for the effects seen in the controlled human exposure studies but do not provide evidence to support a particular quantitative standard.

There is emerging evidence from the controlled human exposure and epidemiologic studies that asthmatics and persons with allergies have stronger response to allergens following exposure to ozone. There is little evidence from either the controlled human exposure or the epidemiologic studies that children are more susceptible than adults to ozone exposure.

The Document indicates that a margin of safety for ozone is based on: (1) chamber studies indicating variability in human response and the existence of particularly large individual responses, (2) chamber studies indicating at higher ozone levels, both bronchial responsiveness and pulmonary inflammation, (3) animal toxicology studies supporting many of these findings and also suggesting the possibility of decreases in lung defense mechanism, and (4) epidemiologic studies reporting associations between ambient ozone and a suite of adverse outcomes including premature mortality, hospitalization, emergency room visits, school loss, respiratory symptoms and changes in lung function. The Document argues that the margin of safety is adequate. We agree that the difference between where effects have been reported and the proposed standards is relatively small. However, the document would benefit greatly from a more thorough discussion of what the margin-of-safety is and how it was derived.

The Document would also benefit considerably from a more critical examination of the literature, specifically of the chamber and epidemiological studies, their limitations, and the applicability of the data to setting a general population standard.

REFERENCES

Adams WC. Comparison of chamber and face-mask 6.6 hour exposures to ozone on pulmonary function and symptoms responses. *Inhal Toxicol* 2002;14:745-64.

American Thoracic Society. What constitutes an adverse health effect of air pollution? *Am J Respir Crit Care Med* 2000;161: 665-673.

Catalano PJ, Rogus J, Ryan M. Consequences of prolonged inhalation of ozone on F344/N rats: collaborative studies. Part X: Robust composite scores based on median polish analysis. *Res Rep Health Eff Inst* (65 Pt 10):1-57; discussion 59-64, 1995.

Clyde M. Model uncertainty and health effect studies for particulate matter. *Environmetrics* 2000;11:745-63.

Dockery DW, Pope CA III, Xu X, et al. An association between air pollution and mortality in six U.S. cities. *N Engl J Med* 1993;329:1753-59.

Folinsbee LJ, Horstman DH, Kehrl HR, et al. Effects of single and repeated prolonged low-level ozone exposure in man. Presented at the annual meeting of the Society of Occupational and Environmental Health, Washington, DC, March 1991.

Frischer T, Studnicka M, Gartner C, et al. Lung function growth and ambient ozone: a three-year population study in school children. *Am J Respir Crit Care Med* 1999;160:390-96.

Gauderman WJ, Gilliland GF, Vora H, et al. Association between air pollution and lung function growth in southern California children: results from a second cohort. *Am J Respir Crit Care Med* 2002;166:76-84.

Gauderman WJ, McConnell R, Gilliland F, et al. Association between air pollution and lung function growth in southern California children. *Am J Respir Crit Care Med* 2000;162:1383-90.

Gent JF, Triche EW, Holford TR, et al. Association of low-level ozone and fine particles with respiratory symptoms in children with asthma. *JAMA* 2003;290:1859-67.

Gong H Jr, Bradley PW, Simmons MS, et al. Impaired exercise performance and pulmonary function in elite cyclists during low-level ozone exposure in a hot environment. *Am Rev Respir Dis* 1986;134:726-33.

Harkema JR, Plopper CG, Hyde DM, et al. Response of macaque bronchiolar epithelium to ambient concentrations of ozone. *Am J Pathol* 1993;143: 857-66.

Health Effects Institute Special Report: Revised Analyses of Time-Series Studies of Air Pollution and Health. Health Effects Institute, Cambridge, 2003.

Herrington DM and Howard TD. From presumed benefit to potential harm – hormone therapy and heart disease. *N Engl J Med* 2003;349:519-21.

Horstman DH, Folinsbee LJ, Ives PJ, et al. Ozone concentration and pulmonary response relationships for 6.6-hour exposures with five hours of moderate exercise to 0.08, 0.10, and 0.12 ppm. *Am Rev Respir Dis* 1990;142:1158-63.

Koop G and Tole L. Measuring the health effects of air pollution: to what extent can we really say that people are dying from bad air? *J Environ Econ and Management* 2004;47:30-54.

Krewski D, Burnett RT, Goldberg MS, et al. Reanalysis of the Harvard Six Cities study and the American Cancer Society study of particulate air pollution and mortality. A special report of the Institute's Particle Epidemiology Reanalysis Project. Cambridge, MA: Health Effects Institute, 2000.

Kulle TJ, Sauder LR, Hebel JR, et al. Ozone response relationships in healthy nonsmokers. *Am Rev Respir Dis* 1985;132:36-41.

Larson SD, Schelegle ES, Walby WF, et al. Postnatal remodeling of the neural components of the epithelial-mesenchymal trophic unit in the proximal airways of infant rhesus monkeys exposed to ozone and allergen. *Toxicol Appl Pharmacol* 2004;194:211-20.

Last JA, Gelzleichter TR, Harkema J, et al. Consequences of prolonged inhalation of Fischer-344/N rats: collaborative studies. Part I: Content and cross-linking of lung collagen. *Res Rep Health Eff Inst* (65): 1-29; discussion 31-40, 1994.

Lipfert FW, Perry HM Jr, Miller JP, et al. The Washington University-EPRI veterans' cohort mortality study: preliminary results. In: Grant, LD, ed. PM2000: particulate matter and health. *Inhalation Toxicol* 2000;12:41-73.

Lumley T and Sheppard L. Time series analyses of air pollution and health: straining at gnats and swallowing camels? *Epidemiology* 2003;14:13-14.

McConnell R, Berhane K, Gilliland F, et al. Prospective study of air pollution and bronchitic symptoms in children with asthma. *Am J Respir Crit Care Med* 2003;168:790-97.

McConnell R, Berhane K, Gilliland F, et al. Asthma in exercising children exposed to ozone: a cohort study. *Lancet* 2002;359:386-91.

McDonnell WF, Horstman DH, Hazucha MJ, et al. Pulmonary effects of ozone exposure during exercise: dose response characteristics. *J Appl Physiol* 1983;54:1345-52.

McDonnell WF, Horstman DH, Abdul-Salaam S, et al. Reproducibility of individual responses to ozone exposure. *Am Rev Respir Dis* 1985;131:36-40.

McDonnell WF, Kehrl HR, Abdul-Salaam S, et al. Respiratory response of humans exposed to low levels of ozone for 6.6 hours. *Arch Environ Health* 1991;46:145-60.

McDonnell WF, Muller KE, Bromberg PA, et al. Predictors of individual differences in acute response to ozone exposure. *Am Rev Respir Dis* 1993;147:818-25.

Molfino NA, Wright SC, Katz I, et al. Effect of low concentrations of ozone on inhaled allergen responses in asthmatic subjects. *Lancet* 1991;338:199-03.

Pinkerton KE, Ménache MG, Plopper CG. Consequences of prolonged inhalation of ozone on F344/N rats: collaborative studies. Part IX: Changes in the tracheobronchial epithelium, pulmonary acinus, and lung antioxidant enzyme activity. *Res Rep Health Eff Inst* 1995 (65 Pt 8-9): 41-98; discussion 99-10.

Pinkerton KE, Weller BL, Ménache MG, et al. Consequences of prolonged inhalation of ozone on F344/N rats: collaborative studies. Part XIII. A comparison of changes in the tracheobronchial epithelium and pulmonary acinus in male rats at 3 and 20 months. *Res Rep Health Eff Inst* 1998 (65): 1-32; discussion 33-7.

Pope CA III, Burnett RT, Thun MJ, et al. Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. *JAMA* 2002;287:1132-41.

Reiser KM, Tyler WS, Hennessy SM, et al. Long-term consequences of exposure to ozone. II. Structural alterations in lung collagen of monkeys. *Toxicol Appl Pharmacol* 1987;89:314-22.

Schelegle ES and Adams WC. Reduced exercise time in competitive simulations consequent to low level ozone exposure. *Med Sci Sports Exerc* 1986;18:408-14.

Schelegle ES, Miller, LA, Gershwin LJ, et al. Repeated episodes of ozone inhalation amplifies the effects of allergen sensitization and inhalation on airway immune and structural development in Rhesus monkeys. *Toxicol Appl Pharmacol* 2003;191:74-85.

Seal E Jr, McDonnell WF, House DE, et al. The pulmonary response of white and black adults to six concentrations of ozone. *Am Rev Respir Dis* 1993;147:804-10.

Szarek JL, Stewart NL, Zhang JZ, et al. Contractile responses and structure of small bronchi isolated from rats after 30 months' exposure to ozone. *Fundam Appl Toxicol* 1995;28:199-08.

Tager IB. Air pollution and lung function growth: is it ozone? *Am J Respir Crit Care Med* 1999;160:387-89.

Tyler WS, Tyler NK, Last JA, et al. Comparison of daily and seasonal exposures of young monkeys to ozone. *Toxicolo* 1988;50:131-144.